EFFECT OF MEMANTINE ON COUGH REFLEX SENSITIVITY: TRANSLATIONAL STUDIES IN GUINEA PIGS AND HUMANS
(REVISION 2)

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Running Title Page

Running title: Effect of memantine on cough reflex sensitivity

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Non-standard abbreviations:
C5, concentration of capsaicin inducing 5 or more coughs; Cmax, the maximum concentration attained following drug administration; NMDA, N-methyl-D-aspartate; T1/2, the time to half maximal drug concentration; Tmax, the time to peak drug concentrations; URI, upper respiratory tract infection.

Recommended section assignment: Gastrointestinal, hepatic, pulmonary, and renal
Abstract

Cough is the most common complaint for which outpatients in the United States seek medical attention and yet available therapeutic options for cough lack proven efficacy and are further limited by safety and abuse liabilities. Thus, safe and effective cough suppressants are needed. Recent preclinical studies described the antitussive effects of memantine, an N-methyl-D-aspartate receptor (NMDAR) channel blocker used in the treatment of Alzheimer's disease. The goals of the present study were to compare the antitussive effects of memantine, dextromethorphan and codeine in guinea pigs, to relate the dose-dependent actions of memantine in these studies to peak plasma concentrations achieved following oral administration, and to provide the first ever evaluation of the antitussive effect of memantine in humans. In guinea pigs, memantine and codeine were comparable in efficacy and potency but both were superior to dextromethorphan in the citric acid cough challenge model. The pharmacokinetic analyses suggest that memantine was active in guinea pigs at micromolar plasma concentrations. Subsequently, fourteen healthy volunteers as well as 14 otherwise healthy adults with acute viral upper respiratory tract infection (URI) underwent capsaicin cough challenges 6 hours after ingestion of memantine, 20 mg, and matched placebo, in a randomized, double-blind, crossover fashion. In healthy volunteers, memantine significantly inhibited cough reflex sensitivity (p=0.034). In subjects with URI, responsiveness to capsaicin was markedly increased, and in these patients, the inhibition of cough reflex sensitivity by memantine relative to placebo did not reach statistical significance (p=0.088). These data support further research to investigate the potential of memantine as a clinically useful antitussive.
Introduction

Cough is among the most common reasons for which patients worldwide seek medical attention (Hsiao et al., 2010; Irwin et al., 2006; Morice et al., 2004). Although most cases represent transient, self-limited, acute cough due to viral upper respiratory tract infection (common cold), a great unmet need exists for the symptomatic treatment of chronic, refractory cough as well as severe, acute cough (Dicpinigaitis, 2011). Currently available non-prescription antitussive agents lack strong proof of efficacy, whereas narcotic cough suppressants are limited by undesirable or intolerable side effects at antitussive doses (Dicpinigaitis, 2011). Hence, a safe, effective, non-narcotic antitussive remains a sought but unattained goal (Morice et al., 2014).

Advances in clinical monitoring and assessment of cough in patients may facilitate the development of new antitussive therapies (Morice et al., 2007; Smith, 2010). These advances include validated methods for evaluating sensitivity to evoked cough in patients. Capsaicin, the pungent extract of red peppers, has been shown in over a quarter-century of clinical experience, to induce cough in a safe, dose-dependent and reproducible manner (Dicpinigaitis and Alva, 2005; Dicpinigaitis, 2003). Thus, capsaicin cough challenge testing has become an important tool in clinical research, allowing for the accurate measurement of the effect of a pharmacological intervention on the sensitivity of the cough reflex (Dicpinigaitis, 2003; Morice et al., 2007; Dicpinigaitis, 2012; Faruqi et al., 2014). The standard end point measured in capsaicin cough challenge testing is the concentration of capsaicin inducing 5 or more coughs \( (C_5) \). In healthy volunteers, this end point has been demonstrated to be highly reproducible, in the short-term \( (20 \text{ minutes to 14 days}) \) and long term \( (\text{months to years}) \) (Dicpinigaitis, 2003).
Comparable evoked cough responses can be studied in animals, thus facilitating translational studies of both initiators of cough and putative antitussive agents.

Based in large part on the results of cough challenge studies, clinical and preclinical observations suggest a primary role for NMDA type glutamate receptors in cough (Kamei et al., 1989; Canning, 2009; Canning and Mori, 2011; Dicpinigaitis et al., 2014a). Dextromethorphan, for example, amongst the most widely used non-prescription drugs for cough suppression in the United States and in many other countries worldwide, works at least in part through its actions at NMDA receptors (Franklin and Murray, 1992; Dicpinigaitis et al., 2014; Faruqi et al., 2014). In studies performed in guinea pigs, we also recently described the antitussive effects of memantine (Smith et al., 2012), a United States Food and Drug Administration (FDA)-approved drug that is prescribed to approximately 1.5 million Alzheimers Disease patients annually in the United States, Japan, France, Germany, Italy, Spain and the United Kingdom (Datamonitor Website, 2014). Available in two forms, extended release (Namenda XR®; 28 mg maximum dose) and immediate release (Namenda®; 20 mg maximum dose), memantine (Namenda®) is a use dependent NMDA receptor/ channel blocker with fast-off kinetics resulting in little NMDA channel block during threshold NMDA receptor activation and unlike dextromethorphan, has no demonstrable affinity for sigma receptors (Kornhuber et al., 1993; Parsons et al., 1999). By virtue of its highly specific “open-block” of the NMDA receptor-channels and absence of sigma receptor interactions, memantine has no significant psychotropic effects and failed to demonstrate abuse liability in animal studies and in clinical use (Vosburg et al., 2005; Chen and Lipton, 2006). Its clinical profile in frail, elderly patients is remarkable for the absence of adverse effects and abuse liability (Parsons et al., 1999; Thomas et al., 2009; Jones, 2010). All
of these characteristics suggest that memantine may have the desired profile of a drug for cough suppression (Canning, 2009).

In the present study, our goals were to generate additional evidence to support the hypothesis that memantine possesses antitussive activity. In guinea pigs, we sought further preclinical evidence for the relative potency and antitussive efficacy of memantine, to compare these actions of memantine when administered orally to the antitussive effects produced by dextromethorphan and codeine, and to relate these pharmacokinetic properties of memantine to its efficacy in cough challenge models. Translating this work to human subjects, we sought to evaluate the effect of a single 20 mg oral dose of memantine on cough reflex sensitivity in two groups: healthy volunteers and otherwise healthy volunteers with acute viral upper respiratory tract infection (URI), or common cold.
Methods

Citric Acid Evoked Coughing in Guinea Pigs

The Johns Hopkins Animal Care and Use Committee approved all experiments performed in this study. Citric acid evoked coughing was studied in awake guinea pigs as previously described (Smith et al., 2012). Male Hartley guinea pigs (Charles River) were placed in a chamber continuously filled with room air. A pressure transducer monitored respiration and cough responses, which were confirmed by microphone (Emka Technologies, Falls Church, VA). After a 5 minute equilibration period, aerosols of citric acid (0.01-0.3M) were delivered to the recording chamber for 5 minutes, with 5 minute intervals in between cumulatively increasing doses. We used citric acid instead of capsaicin in guinea pigs to avoid the profound bronchospasm associated with capsaicin challenges in guinea pigs. We estimated the potency of citric acid in these assays following curve fitting the data from each animal and estimating C5, the concentration of citric acid evoking 5 coughs. The total number of coughs evoked was also recorded. Thirty minutes prior to cough challenges, animals were pretreated with memantine (3, 10 or 30 mg/ kg), dextromethorphan (10 or 30 mg/ kg), codeine (3, 10 or 30 mg/ kg), diphenhydramine (3 mg/ kg) or the vehicle (saline) for these drugs by oral gavage. Each animal was studied just once in this parallel and unpaired group design.

Pharmacokinetics of memantine in guinea pigs

Our rationale for dosing memantine 30 minutes prior to initiating the citric acid challenges in guinea pigs was based in part upon the results of previous pharmacokinetic analyses performed in rats (Morè et al., 2008), but also on our own time course studies with memantine following intraperitoneal (ip) administration. In these preliminary experiments, 10
mg/kg memantine administered ip markedly reduced the cumulative number of coughs evoked by citric acid when evaluated 60 minutes after administration, but was without effect when evaluated 4 hours after administration (citric acid (0.01-0.3M) evoked 16±5 (n=12), 7±3 (n=6; p<0.05) and 15±5 (n=4) cumulative coughs in animals treated with vehicle or 10 mg/kg memantine 1 and 4 hours prior to citric acid challenge, respectively). In an attempt to relate the antitussive effects of memantine to its peak plasma levels in guinea pigs, a pharmacokinetic analysis was performed (PharmaLegacy Laboratories, Shanghai, China). Memantine (3, 5 and 10 mg/kg po) was administered orally to Hartley guinea pigs (286-377 grams) and detected by LC-MS/MS (detection threshold: 1ng/mL) in serum separated from venous blood drawn 0.25, 0.5, 1, 2, 4, 8 and 24 hours after dosing. The antitussive effects of memantine vs. 0.4M citric acid evoked coughing were determined in parallel using methods identical to those described above.

Capsaicin-Evoked Coughing in Human Volunteers

Otherwise healthy adult nonsmokers (Subject group 1) and otherwise healthy nonsmokers with the onset of symptoms consistent with acute viral upper respiratory tract infection (URI) within the previous 72 hours (Subject group 2) were recruited and enrolled after providing informed consent. The study protocol was approved by the Institutional Review Board of Montefiore Medical Center, Bronx, NY. Subjects did not have a history of asthma or other pulmonary disease, nor history or symptoms suggestive of gastroesophageal reflux disease. Subjects presenting with symptoms suggestive of influenza, such as high fever, myalgias, or severe illness, were excluded. Individuals with URI who had taken any cough/cold medicines for their illness were excluded, as were those currently receiving any medication known to affect
cough reflex sensitivity, or who had received any such medication within a time frame that would leave the possibility of a lingering effect of a previously-ingested medication on the sensitivity of the cough reflex.

Upon enrollment, subjects were randomized to receive memantine (Namenda 20 mg) or matched placebo capsules 6 hours prior to capsaicin cough challenge testing, in a double-blind, cross-over fashion. Randomization was arranged so that half of the subjects would receive placebo first, and half would initially receive memantine. Study drugs were administered 6 hours prior to cough challenge testing to provide near-peak blood levels at time of challenge, demonstrated to be approximately 60 ng/ml in other studies (Periclou et al., 2006; FDA CDER Website, 2014). The two cough challenge studies were performed 4-7 days apart so as to capture the acute phase of URI, and to allow for at least partial washout of study drug.

Capsaicin cough challenge was performed as previously described (Dicpinigaitis, 2003; Morice et al., 2007). Briefly, subjects inhaled single, vital-capacity breaths of ascending, doubling concentrations (range 0.49 μM to 1,000 μM) of aerosolized capsaicin solution, administered via a compressed air-driven nebulizer controlled by a dosimeter, with 1-minute intervals between inhalations, until 5 or more coughs resulted in the 15 seconds following an inhalation. Placebo saline breaths were randomly interspersed between capsaicin doses to increase challenge blindness. The end point of capsaicin challenge testing is the concentration of capsaicin inducing 5 or more coughs (C₅).

Statistical Analyses

Cough responses to citric acid in guinea pigs and the pharmacokinetic parameters measured for memantine are presented as the mean ± sem of n experiments, where n is a single
animal evaluated in parallel, nonpaired experimental designs. Differences amongst group means were evaluated by analysis of variance, and treatment means were compared to control in post-hoc analyses by Dunnett’s multiple comparison tests. Evidence of dose-dependent effects of memantine in guinea pigs were evaluated by linear regression. Cough patterns were compared amongst treatment groups by Chi square analysis. A p-value of less than 0.05 was considered statistically significant (Graphpad Prism 6).

Based on previous work by one of the authors determining reproducibility of serial capsaicin cough challenges in humans, a subject number of 14 has been demonstrated to be adequate to evaluate the effect of a study drug vs. placebo in a cross-over protocol (Dicpinigaitis & Gayle, 2003). Changes in post-placebo and post-memantine cough reflex sensitivity measurements were compared by paired t-test.

Reagents

For the animal studies, citric acid, memantine, diphenhydramine and codeine were purchased from Sigma (St. Louis, MO). Dextromethorphan was purchased from MP Biomedicals (Solon, Ohio). Citric acid was dissolved in water. All putative antitussive drugs administered to guinea pigs were dissolved in saline at concentrations suitable for achieving target doses with 1 mL administered orally by gavage/ 100 mg body weight.

The human studies used commercially-available memantine (Namenda, Forest Laboratories, New York, USA) and pharmaceutical-grade capsaicin (Formosa Labs, Taiwan, ROC).
Results

Citric acid-evoked coughing in guinea pigs

Citric acid aerosols evoked coughing dose-dependently in awake guinea pigs. In untreated control animals 0.01, 0.1 and 0.3M citric acid evoked coughing in 2, 8 and 15 out of 15 separate experiments, respectively. Overall, citric acid evoked 18±3 coughs cumulatively in control animals (n=15; range: 1 – 31). Occasionally, the coughing became repetitive and even paroxysmal in pattern. This manifested as a cumulative cough total of 15 or more coughs, which was observed in 8 of 15 control animals, with 5 of 15 control animals producing 28 or more coughs in response to citric acid.

At a dose of 10 mg/ kg (n=9), orally administered memantine significantly reduced citric acid evoked coughing (Figure 1). A dose of 30 mg/ kg po memantine virtually abolished the cough responses (n=5). Comparable results were obtained with codeine (3-30 mg/ kg po; n=5-8). By contrast, orally administered dextromethorphan (10 and 30 mg/ kg po; n=5-8) was less effective than codeine or memantine at preventing citric acid evoked coughing.

When administered at doses of 10 mg/ kg, memantine, codeine and dextromethorphan did not alter the threshold sensitivity to citric acid (–log C5 values averaging 0.98±0.1, 0.77±0.06 and 0.87±0.1 and 0.83±0.1 in animals treated with vehicle, memantine, codeine or dextromethorphan, respectively (n=5-14; p>0.05)). At 30 mg/ kg, however, each of these drugs markedly reduced both the potency and efficacy of citric acid to evoke coughing, with 60% (dextromethorphan), 80% (memantine) and 100% (codeine) of animals failing to cough 5 or more times (n=5/ treatment group), while only 1 out of 15 (<7%) control animals failed to cough 5 or more times in response to citric acid. None of these treatments were associated with any overt signs of respiratory depression, sedation or abnormal behavior.
In pharmacokinetic analyses, peak plasma levels following oral administration of 10 mg/kg memantine reached 817±213 ng/mL (~4.5µM) and were attained within 15-60 minutes of administration in 4 of 6 guinea pigs studied (Table 1). Lower doses of memantine (3 and 5 mg/kg po) achieved peak plasma levels of 170±20 and 290±57 ng/mL, respectively (1-2 µM; n=6 each). Consistent with previous studies performed in rats and mice (Morè et al., 2008; Beconi et al., 2011), the T_{1/2} for memantine in guinea pigs was achieved between 4 and 7 hours following oral administration. By 24 hours, memantine (administered at 3, 5 and 10 mg/kg po) was still detectable in guinea pig plasma (5±5, 6±4 and 29±12 ng/mL, respectively), but at levels less than 10% of C_{max} (range: 0.2-9%).

In parallel with these pharmacokinetic analyses, the potency and efficacy of orally administered memantine against coughing evoked by a single challenge with 0.4M citric acid aerosols was determined (figure 2). In control animals, 0.4M citric acid evoked 17±3 coughs. Memantine dose-dependently (1-30 mg/kg po) inhibited 0.4M citric acid evoked coughing ($r^2 = 0.26$, p<0.001) and a post-hoc analysis detected statistically significant reductions in citric acid evoked coughing in animals treated with 10 and 30 mg/kg memantine (n=10/treatment group). We also studied the synergistic effects of memantine when co-administered with diphenhydramine, the 1st generation antihistamine with antitussive effects in guinea pigs (McLeod et al., 1998) and a frequent combination therapy with dextromethorphan in over the counter cough medicines (FDA advisory committee, 2010). When administered orally at 3 mg/kg and 10 mg/kg, respectively, neither memantine nor diphenhydramine significantly inhibited cumulative (0.01 – 0.3M) citric acid evoked coughing in guinea pigs (14±2 (n=17), 10±2 (n=12) and 13±3 (n=4)) coughs evoked cumulatively by citric acid (0.01-0.3M) in control animals and animals pretreated with 3 mg/kg memantine or 10 mg/kg diphenhydramine, respectively.
When co-administered, however, the combination of memantine and diphenhydramine markedly reduced citric acid evoked coughing (5±3 coughs; n=7; p<0.05).

Capsaicin-evoked coughing in humans

Fourteen healthy volunteers (7 female; mean age 37.4 yr) were enrolled and completed the study. Mean log C5 was 0.71±0.15(SEM) after placebo, and 0.96±0.12 after memantine (p=0.034), indicating an inhibition of cough reflex sensitivity to inhaled capsaicin (Figure 3). Five subjects reported side effects after memantine, including: mild lightheadedness in two subjects; moderate sleepiness in one subject; mild dizziness in one subject; and, mild headache and nausea in one subject. One subject reported a relaxed feeling after placebo. In all subjects, side effects were transient, reached peak severity 1-3 hours after drug ingestion, and did not preclude completion of the study.

Fourteen subjects with URI (12 female; mean age 39.2 yr) completed the study. Mean log C5 was 0.26±0.09 after placebo, and 0.43±0.08 after memantine (p=0.088; Figure 4). Within this group, one subject reported mild dizziness after memantine, and, after placebo, one subject reported a feeling of jitteriness and brief palpitations, whereas another subject after placebo reported abdominal cramping and headache. One subject withdrew from the study before cough challenge testing due to severe dizziness occurring after ingestion of memantine.

Of note, we observed no correlation between the occurrence of side effects and the inhibition of cough reflex sensitivity by memantine.
Discussion

Cough is initiated by activation of bronchopulmonary vagal afferent nerves, which utilize glutamate to transduce excitatory synaptic input at their central terminations in the brainstem (Dicpinigaitis et al., 2014a; Canning, 2009; Canning et al., 2014). The excitatory effects of glutamate are dependent upon the activation of the ionotropic NMDA and non-NMDA type glutamate receptors. NMDA receptors at rest are unable to fully transduce glutamate-induced actions due to a persistent block of their channel pores by magnesium ions. Only with sustained excitatory input and depolarization that relieves magnesium block are NMDA receptors capable of fully transducing synaptic transmission (Chen and Lipton, 2006). These pharmacological properties of NMDA receptors predict their involvement in cough. Thus, induced cough in humans is preceded by an initial urge to cough (Davenport et al., 2007; Dicpinigaitis et al., 2012), an observation that is likely the clinical manifestation of the physiologic evidence that cough initiation requires sustained, high frequency vagal afferent nerve activation in animals (Canning, 2009; Canning and Mori, 2011). Consistent with the involvement of NMDA receptors in cough, both ketamine and dextromethorphan have been reported to suppress cough in patients (Yeh et al., 2007; Faruqi et al., 2014). Moreover, in preclinical studies, dextromethorphan and other NMDA receptor/channel blockers prevent evoked coughing in dogs, cats, rabbits, guinea pigs and rats (Kamei et al., 1989; Dicpinigaitis et al., 2014a; reviewed in Canning, 2009; Canning and Mori, 2011). Further supporting a role for NMDA receptors in cough, here and previously (Smith et al., 2012) we observed that the NMDA receptor channel blocker memantine prevents evoked coughing in guinea pigs. We have also reported here that memantine reduced sensitivity to capsaicin evoked coughing in human subjects.
The need for sustained, high frequency vagal afferent nerve activation and the involvement of NMDA receptors in the central encoding of cough has both pathophysiological and therapeutic implications (Canning et al., 2014; Dicpinigaitis et al., 2014). In acute and chronic conditions associated with excessive coughing and enhanced cough reflex sensitivity, for example, inflammation that increases vagal afferent nerve excitability or recruited synaptic inputs that enhance synaptic efficacy at the central terminations of bronchopulmonary vagal afferent nerves may result in cough hypersensitivity and perhaps excessive coughing provoked by only mildly irritating stimuli (e.g. cold air, perfumes) or even to otherwise innocuous stimulants (Morice et al. 2014). Such cough hypersensitivity was apparent in the present study in those patients with URIs, with capsaicin C₅ values 2-3 fold lower than those observed in healthy subjects, which is consistent with transient, viral URI-induced cough reflex hypersensitivity reported in previous studies (Dicpinigaitis et al., 2014b). Restoring normal cough reflex sensitivity and thus suppressing the excessive coughing associated with disease does not require complete inhibition of vagal afferent nerve activation or a complete suppression of synaptic transmission at the central terminations of the vagal afferents, just a reduction of afferent drive and/ or a modest reduction in central synaptic efficacy (Canning, 2009; Canning et al., 2014).

Despite the clinical and preclinical evidence suggesting a role for NMDA receptor blockade in cough suppression, there are considerable challenges associated with such a therapeutic strategy. Both dextromethorphan and ketamine have received considerable attention of late for their safety and abuse liabilities, particularly in children (FDA advisory committee, 2010; DeLuca et al., 2012; Cooper, 2013). Off target effects such as Sigma receptor activation by dextromethorphan (Franklin and Murray, 1992; Fontanilla et al., 2009) and HCN1 channel
blockade by ketamine (Chen et al., 2009) likely contribute to these undesirable effects and are dose-limiting, especially in patients seeking relief from cough. But even highly selective NMDA receptor/channel ligands may be difficult to develop as cough therapies. NMDA receptors subserve homeostatic as well as pathophysiologic functions, thus any attempt at manipulating NMDA receptor function for cough suppression may be hindered to some extent by side effects associated with preventing their homeostatic actions (Chen and Lipton, 2006). Ideally, a therapeutic strategy selectively targeting the excessive and pathophysiologic gating of NMDA receptors/channels or a drug that selectively targeted the NMDA receptor subtypes localized to neurons relevant to cough transduction would be desirable (Chen and Lipton, 2006; Paoletti and Lipton, 2007; Smith et al., 2012). Memantine, a use dependent NMDA receptor channel blocker, would appear to have such properties. With its open channel blocking actions and rapid dissociation rates from the channel complex (Kotermanski et al., 2009), memantine displays the peculiar in vitro characteristic of being more effective at preventing receptor/channel gating by high concentrations of NMDA receptor agonists as compared to its actions on threshold NMDA receptor activation (Chen et al., 1992). With these highly specific actions and absence of sigma receptor engagement, memantine has no significant psychotropic effects and failed to demonstrate abuse liability in animal studies or in clinical use (Kornhuber et al. 1993; Parsons et al., 1999; Vosburg et al., 2005; Chen and Lipton, 2006). Clinical observations would seem to support the in vitro pharmacological attributes described above. In multiple studies memantine provides therapeutic benefit in patients with moderate to severe dementia while having few if any undesirable side effects (Thomas and Grossberg et al., 2009; Jones, 2010). All of these properties predicted the potential utility of memantine in cough, a prediction that was satisfied in studies performed in guinea pigs, in which memantine suppressed cough evoked by either citric
acid or bradykinin inhalation, while displaying none of the undesirable side effects observed in parallel studies with dextromethorphan and ketamine (Canning, 2009; Smith et al., 2012). We have confirmed and extended these previous studies here by documenting the antitussive effects of memantine when administered orally. We observed that memantine was superior or equally effective at suppressing cough in guinea pigs when compared to either dextromethorphan or codeine.

Although the pharmacokinetics of memantine in small animals (present study; Morè et al., 2008; Beconi et al., 2011) differs from that reported in patients (Periclou et al., 2006; Grossberg et al., 2013), such studies are informative as they allow direct comparisons of efficacy with estimated concentrations in the relevant biophase and with known affinities of memantine for NMDA receptor channels. Regarding the latter, in vitro assessments of memantine potency have produced IC₅₀ values that vary widely but have generally been upwards of 0.1 µM (Chen et al. 1992; Kotermanski et al. 2009). In our pharmacokinetic analyses, peak plasma levels of memantine ranged between 1 and 10 µM when dosed orally at 3-10 mg/ kg. Only when dosed at 10 and 30 mg/ kg and studied 30 to 60 minutes after dosing, when peak plasma levels were expected, did memantine consistently reduce citric acid evoked coughing. That does not mean that lower doses of memantine are biologically inactive. A linear relationship between dose and cough suppression was apparent over doses ranging from 1-30 mg/ kg. We also observed that while 3 mg/ kg memantine failed to significantly inhibit citric acid evoked cough, this dose of memantine worked synergistically with diphenhydramine to markedly reduce coughing. This synergistic effect may have important therapeutic implications in patients, who often take multiple drugs to treat either acute cough or drugs to treat chronic conditions resulting in cough (e.g. antihistamines for rhinosinusitis).
Antitussive effects of memantine were also demonstrated in healthy volunteers by its ability to reduce cough reflex sensitivity to inhaled capsaicin. In subjects with URI, although 8/14 patients were less sensitive to capsaicin when receiving memantine, the degree of suppression of cough reflex sensitivity by memantine relative to placebo did not reach statistical significance (p=0.088). The single, 20 mg oral dose of memantine was fairly well tolerated, without apparent correlation between the experience of adverse effects and cough suppression.

It is noteworthy that memantine reduced cough reflex sensitivity in healthy subjects after a single 20 mg dose, a dose that is provided daily and chronically to patients with dementia. As mentioned above, this single dose of memantine achieves a $C_{\text{max}}$ of approximately 60 ng/mL (~300nM), a concentration that is 2-20 times lower than most reported IC$_{50}$ values for memantine in in vitro studies (Parsons et al., 1999) and more than 10 times lower than the peak plasma levels achieved by the consistently antitussive dose of memantine (10 mg/kg) evaluated in guinea pigs. Far greater cough suppression would be expected with higher doses of memantine or with sustained dosing, where plasma concentrations progressively increase for the slowly cleared drug (Periclou et al., 2006; Grossberg et al., 2013). Results from studies performed in patients with dementia suggest this would not be associated with any excessive and undesirable side effects (Thomas and Grossberg et al., 2009; Jones, 2010), although this would have to be evaluated more carefully in patients with cough.

Among the limitations of this exploratory study is its small size. If powered comparable to our animal studies, it is conceivable that more compelling evidence for cough suppression would have been apparent in both clinical studies. Furthermore, the washout period of 4-7 days used in this study represented less than 5 half lives of the study drug, given the half life of immediate-release memantine of 60 hours (Periclou et al., 2006; Grossberg et al., 2013; FDA
CDER Website, 2014). Therefore, those subjects having been randomized to receive memantine first in this cross-over trial may still have had significant levels of memantine present at the time of their post-placebo cough reflex sensitivity measurement, thus potentially blunting the difference between the effects of memantine and placebo. The washout period was chosen so as to ensure stability of the cough reflex during the study period, as it has been demonstrated that capsaicin cough reflex sensitivity (C₃) remains stable during the first week of URI (Dicpinigaitis et al., 2014b), but stability beyond this time frame has not been confirmed. Despite these shortcomings, this study further substantiates the rationale for NMDA receptor/ channel blockade in cough. Whether therapeutic efficacy can be achieved in the absence of unwanted side effects awaits further analysis. Overall, however, further evaluation of memantine as a potentially safe and effective antitussive agent appears warranted.
Authorship Contributions

Participated in research design: Dicpinigaitis, Canning, Paterson

Conducted experiments: Dicpinigaitis, Canning

Performed data analysis: Dicpinigaitis, Canning, Garner, Paterson

Wrote or contributed to writing of manuscript: Dicpinigaitis, Canning, Garner, Paterson
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Footnotes

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Disclosure:

BP is CEO of Cerecor, Inc. RG is Director, Translational Medicine, Cerecor, Inc. PVD and BJC serve on the Developmental Advisory Board of Cerecor, Inc.
Suppression of citric acid evoked coughing in awake guinea pigs by memantine, codeine and dextromethorphan (n=5-14). Drugs were administered orally 30 minutes prior to citric acid (0.01-0.3M) challenge at A and C) doses of 10 mg/ kg, and B and D) 30 mg/ kg. The results are presented as A and B) the mean±sem coughs evoked cumulatively, and C and D) the percentage of animals coughing 15 or more time in response to citric acid. An asterisk (*) indicates values that are statistically different when compared to control values.

Dose-dependent effects of orally administered memantine on 0.4M citric acid evoked coughing (n=10/ treatment group). There was a linear relationship between memantine dose and the total number of coughs evoked (r2 = 0.26; p<0.001). An asterisk (*) indicates values that are statistically different when compared to control values.

Cough reflex sensitivity measurements in 14 healthy volunteers. Mean log C5 was 0.71±0.15(SEM) after placebo, and 0.96±0.12 after memantine (p=0.034), indicating an inhibition of cough reflex sensitivity to inhaled capsaicin. C5=the concentration of capsaicin inducing ≥5 coughs. Error bars represent ±SEM.

Cough reflex sensitivity measurements in 14 otherwise healthy subjects with acute viral upper respiratory tract infection (URI). Mean log C5 was 0.26±0.09 after placebo, and 0.43±0.08 after
memantine (p=0.088). $C_5$=the concentration of capsaicin inducing ≥5 coughs. Error bars represent ±SEM.
Table 1. Pharmacokinetics of orally administered memantine in guinea pigs

<table>
<thead>
<tr>
<th>Time course of memantine plasma concentrations (ng/ mL)</th>
<th>3 mg/ kg</th>
<th>5 mg/ kg</th>
<th>10 mg/ kg</th>
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<td>0.25 hours</td>
<td>84±31</td>
<td>189±76</td>
<td>735±247</td>
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<td>0.5 hours</td>
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</tr>
<tr>
<td>8 hours</td>
<td>41±13</td>
<td>67±14</td>
<td>184±29</td>
</tr>
<tr>
<td>24 hours</td>
<td>5±4</td>
<td>6±4</td>
<td>34±12</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 mg/ kg</th>
<th>5 mg/ kg</th>
<th>10 mg/ kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ mL)</td>
<td>176±17</td>
<td>290±57</td>
<td>817±213</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hours)</td>
<td>1±0.22</td>
<td>0.83±0.27</td>
<td>1.29±0.61</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (hours)</td>
<td>4.76±1.42</td>
<td>4.23±0.72</td>
<td>6.29±0.8</td>
</tr>
</tbody>
</table>

These data are presented as a mean ± sem of 6 experiments. Memantine was measured in plasma collected from venous blood. See the methods section for further details.
Cumulative Number of Coughs

Control
Memantine
Codeine
DXM

Figure 1A

10 mg/kg

*
Figure 1B

Cumulative Number of Coughs

Control  Memantine  Codeine  DXM

30 mg/kg
Figure 1C

% Animals Coughing ≥15 Times

10 mg/kg

Control Memantine Codeine DXM
Figure 4

The graph shows the log concentration of $C_5$ (in μM) for placebo and memantine treatments. The data points are connected by lines, and error bars are included to indicate variability. The x-axis represents placebo and memantine treatments, while the y-axis represents the log concentration of $C_5$. The graph suggests a trend where the log concentration of $C_5$ is lower in the memantine treatment compared to the placebo.